

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

SCHENK, Dale B.

Application No.: 09/724,319

Filed: November 27, 2000

For: PREVENTION AND TREATMENT
OF AMYLOIDOGENIC DISEASE

Confirmation No.: 6653

Examiner: K. Ballard, Ph.D.

Art Unit: 1649

DECLARATION OF
DR. PETER SEUBERT
UNDER 37 C.F.R. §1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Peter Seubert, state as follows:

1. My current position is Vice President, Neurodegenerative Research at Elan Pharmaceuticals, Inc., the assignee of the above-captioned application. A copy of my curriculum vitae is attached.

2. I understand that Solomon, US Patent No. 5,688,651 reports that an antibody designated AMY-33 has a putative epitope between residues 25-28 of A β . I understand this putative assignment is based on AMY-33 being raised against a 1-28 fragment of A β , and Yankner et al., Science 250:279-282 (1990) having speculated that residues 25-35 of A β mediated toxic effects. The putative epitope 25-28 represents the intersection of the fragment generating AMY-33 with the 25-35 toxic region hypothesized by Yankner et al.

3. I or others acting under my supervision have performed the following experiment to determine the epitope specificity of the AMY-33 antibody. We obtained the antibody from Zymed Laboratories, Inc (now Invitrogen).

4. The following peptides were used for analysis. A β 1-10 C-terminally biotinylated obtained from Mimotopes; A β 1-16 N-terminally biotinylated obtained from Anaspec; A β 13-28 biotinylated through a C-terminal cysteine using Pierce's no weigh maleimide PEO2-Biotin at a 10 M ratio; and, A β 1-38 biotinylated using Pierce's NHS PEO2 Biotin at a 5 M ratio following ForteBio's recommended biotinylation protocol. Binding was detected using a ForteBio's Octet, which is a label-free instrument for determining both binding and kinetics of protein-protein interactions. For this experiment, the ForteBio Strepavidin High binding FA Biosensors were loaded with 5 μ g/ml of the different biotinylated A β peptides. The sensors were then allowed to reach baseline before the association and dissociation and K_D of each antibody was determined. Antibodies were run at molar concentrations of 1-10 times their expected K_D . Various antibodies whose epitope specificity had previously been determined, specifically 2H3 (epitope =A β 2-7) and 12A11 (epitope =A β 3-7), were used as a positives control for various n terminal peptides and A β 1-38: antibody 266 (epitope =A β 16-23) was used as the positive control for 13-28 and 6H9(epitope A β 19-22) was used as the positive control for A β 17-28.

5. The table below shows the measured affinity of AMY33 for the various fragments of A β tested. In brief, AMY33 showed binding to A β 1-10, 1-16 and 1-38 at approximately 100 fold less affinity than the control antibody 12A11 but showed no detectable binding to A β 13-28 and 17-28.

		BINDING AFFINITY				
		K _D Aβ 1-10	K _D Aβ 1-16	K _D Aβ 1-38	K _D Aβ 13-28	K _D Aβ 17-28
ANTIBODY	AMY 33	100-300 nM	100-300 nM	100-300 nM	No binding	No binding
	12A11	Not done	1-2 nM	1-3 nM	No binding	No binding
	2H3	0.6 nM	0.2 nM	0.45 nM	Not done	Not done
	266	Not done	Not done	Not done	0.15 nM	Not done
	6H9	Not done	Not done	Not done	0.6 nM	Not done

6. I conclude from the detectable binding to Aβ1-10 and lack of detectable binding to the Aβ13-28 fragment that the epitope bound by AMY-33 does not lie within residues 13-28 of Aβ.

7. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,


Peter Seubert

Date: October 11, 2007

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EDUCATION

- 1984 **Ph.D., Biochemistry**, University of California, Davis
Dissertation Title: Functional studies of ATP sulfurylase from *Penicillium chrysogenum*
Advisor: Dr. Irwin H. Segel
- 1979 **B.S., Biochemistry**, University of California, Davis
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SCIENTIFIC BACKGROUND

- 1989-present **Vice President Biology Research**, Elan Pharmaceuticals, South San Francisco, California.
Discovery and development of biochemical tests for the diagnosis of Alzheimer's disease. Studies of the metabolism and aberrant deposition of the amyloid protein and microtubule associated protein tau.
Characterization of inhibitors of amyloid formation in neuronal culture and transgenic mouse model of Alzheimer's disease. Development of vaccine-based approach to Alzheimer's disease treatment.
- 1988-1989 **Scientific Consultant**, Cortex Pharmaceuticals, Inc., Irvine, California.
- 1985-1989 **Postdoctoral Fellow and Assistant Researcher**, Bonney Center for the Neurobiology of Learning and Memory, University of California, Irvine; laboratory of Dr. Gary Lynch. Investigation of the role of calcium-activated proteases in memorial and pathological neuronal events. Demonstration of intracellular proteolysis of brain spectrin coupled to activation of NMDA receptors during ischemia and in response to toxins, lesions, and in certain hereditary disorders. Biochemical studies of roles of calcium activated proteins (calpain, protein kinase C, calmodulin) in neuronal plasticity and degeneration.

proteins (calpain, protein kinase C, calmodulin) in neuronal plasticity and degeneration.

- 1979-1984 **Graduate Student**, Department of Biochemistry and Biophysics, University of California, Davis. Studies of sulfate activating enzymes: ATP sulfurylase and adenosine 5'-phosphosulfate kinase. Initial velocity, product inhibition, inhibition by substrate analogs, analysis of reaction progress curves by a simplified integrated rate equation, alternative substrates, and equilibrium binding studies used to deduce kinetic mechanisms and ligand binding order of the sulfate activating enzymes.
- 1978-1979 **Undergraduate Student**, Department of Biochemistry and Biophysics, University of California, Davis; under direction of Dr. Irwin H. Segel. Isolation and preliminary characterization of ATP sulfurylase from mammalian source and thermophilic fungus for comparative studies with mesophilic fungus enzyme.
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AWARDS AND HONORS

- National Institute on Aging Postdoctoral Training Fellowship 1985
 - Jastro-Shields Research Scholarship Awards 1978, 1979, 1982, 1983
 - University of California Regents' Fellowship 1982
 - Earl C. Anthony Fellowship 1981
 - Outstanding Teaching Assistant in Biochemistry Award 1981
 - Henry A. Jastro Scholar in Biochemistry Award 1980
 - Andrew Christensen Scholarship 1979
 - Outstanding Undergraduate Achievement in Biochemistry Citation 1979
 - Graduation with high honors, University of California, Davis 1979
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PUBLICATIONS

1. Farley, J.R., Christie, E.A., Seubert, P.A., and Segel, I.H. (1979) Adenosinetriphosphate sulfurylase from *Penicillium chrysogenum*: Evidence for essential arginine, histidine and tyrosine residues. J. Biol. Chem., 254:3537-3542.
2. Seubert, P.A., Grant, P.A., Christie, E.A., Farley, J.R., and Segel, I.H. (1980) Kinetic and chemical properties of ATP sulphurylase from *Penicillium chrysogenum*. In: Sulphur in Biology, Ciba Foundation Symposium, 72:19-47.
3. Seubert, P.A., Hoang, L., Renosto, F., and Segel, I.H. (1983) ATP sulfurylase from *Penicillium chrysogenum*: measurements of the true specific activity of an enzyme subject to potent product inhibition and reassessment of the kinetic mechanism. Arch. Biochem. Biophys., 225:679-691.
4. Renosto, F., Seubert, P.A., and Segel, I.H. (1984) Adenosine 5'-phosphosulfate kinase from *Penicillium chrysogenum*: purification and kinetic characterization. J. Biol. Chem., 259:2113-2123.
5. Renosto, F., Seubert, P.A., Knudson, P., and Segel, I.H. (1985) APS kinase from *Penicillium chrysogenum*: dissociation and reassociation of subunits as the basis of the reversible heat inactivation. J. Biol. Chem., 260:1535-1544.
6. Seubert, P.A., Renosto, F., Knudson, P., and Segel, I.H. (1985) Adenosinetriphosphate sulfurylase from *Penicillium chrysogenum*: steady-state kinetics of the forward and reverse reactions, alternate substrate kinetics, and equilibrium binding studies. Arch. Biochem. Biophys., 240:509-523.
7. Segel, I.H., Renosto, R., and Seubert, P.A. (1987) The sulfate activating enzymes. Methods in Enzymology, 143:333-349.
8. Seubert, P., Baudry, M., Dudek, S. and Lynch, G. (1987) Calmodulin stimulates the degradation of brain spectrin by calpain. Synapse, 1:20-24.
9. Baudry, M., Seubert, P. and Lynch, G. (1987) A possible second messenger system for the production of long-term changes in synapses. In: Molecular Mechanisms of Neuronal Responsiveness (Y.H. Ehrlich, R.H. Lenox, E. Kornbeck, W.O. Berry, ed.). Plenum Press, New York and London, 291-311.

10. Perlmuter, L., Siman, R., Gall, C., Seubert, P., Baudry, M. and Lynch, G. (1988) Ultrastructural localization of calcium-activated proteases (calpain) in rat brain. *Synapse*, 2:79-88.
11. Ivy, G.O., Seubert, P., Baudry, M. and Lynch, G. (1988) Presence of brain spectrin in dendrites of mammalian brain: technical factors involved in immunocytochemical detection. *Synapse*, 2:329-333.
12. Seubert, P., Ivy, G., Larson, J., Lee, J., Shahi, K., Baudry, M. and Lynch, G. (1988) Lesions of entorhinal cortex produce a calpain-mediated degradation of brain spectrin in dentate gyrus. I. Biochemical studies. *Brain Res.*, 459:226-233.
13. Ivy, G., Seubert, P., Lynch, G. and Baudry, M. (1988) Lesions of entorhinal cortex produce a calpain-mediated degradation of brain spectrin in dentate gyrus. II. Anatomical studies. *Brain Res.*, 459:233-241.
14. Seubert, P., Larson, J., Oliver, M., Jung, M.W., Baudry, M. and Lynch, G. (1988) Stimulation of NMDA receptors induces proteolysis of spectrin in hippocampus. *Brain Res.*, 460:189-195.
15. Lynch, G., Muller, D., Seubert, P., and Larson, J. (1988) Long-term potentiation: persisting problems and recent results. *Brain Res. Bull.*, 21:363-372.
16. Lynch, G., Seubert, P. (1989) Links between long-term potentiation and neuropathology: an hypothesis involving calcium-activated proteases. *Annals of the New York Academy of Sciences*, Vol. 568. In: Calcium, Membranes, Aging, and Alzheimer's Disease (Khachaturian, A.S. Cotman, C.W., and Pettegrew, J.W., eds.) New York Academy of Sciences, NY, 568:171-180.
17. Seubert, P., Nakagawa, Y., Ivy, G., Vanderklish, P., Lynch, G., and Baudry, M. (1989) Intrahippocampal colchicine injection results in spectrin proteolysis. *Neurosci.* 31:195-202.
18. Seubert, P., Lee, K., and Lynch, G. (1989) Ischemia triggers NMDA receptor-linked cytoskeletal proteolysis in hippocampus. *Brain Res.*, 492:366-370.
19. Seubert, P., Peterson, C., Vanderklish, P., and Lynch, G. (1990) Elevated levels of brain spectrin proteolysis in the Brindled mouse. *Neurosci. Lett.* 108:303-308.
20. Seubert, P. and Lynch, G. (1990) Plasticity to pathology: brain calpains as modifiers of synaptic structure. In: *Intracellular calcium-dependent*

- proteolysis (R.L. Mellgren and T. Murachi, eds.) CRC Press, Boca Raton, 251-264.
21. Sinha, S., Dovey, H.F., Seubert, P., Ward, P.J., Blacher, R.W., Blaber, M., Bradshaw, R.A., Arici, M., Mobley, W.C., and Lieberburg, I. (1990) The protease inhibitory properties of the Alzheimer's β -amyloid precursor protein. *J. Biol. Chem.* 265:8983-8985.
 22. Peterson, C., Vanderklis, P., Seubert, P., Cotman, C. and Lynch, G. (1991) Increased spectrin proteolysis in fibroblasts from aged and Alzheimer donors. *Neurosci. Lett.* 121:239-243.
 23. Schenk, D., Seubert, P., Johnson-Wood, K., Lieberburg, I., (1991) Biochemical markers for Alzheimer disease. *Bull. Clin. Neurosci.* 56:161-167.
 24. Seubert, P., Vigo-Pelfrey, C., Esch, F., Lee, M., Dovey, H., Davis, D., Sinha, S., Schlossmacher, M., Whaley, J., Swindlehurst, C., McCormack, R., Wolfert, R., Selkoe, D., Lieberburg, I., Schenk, D. (1992) Isolation and quantitation of soluble Alzheimer's beta-peptide from biological fluids. *Nature* 359:325-327.
 25. Citron, M., Oltersdorf, T., Haass, C., McConlogue, L., Hung, A.Y., Seubert, P., Vigo-Pelfrey, C., Lieberburg, I., Selkoe, D.J. (1992) Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production. *Nature* 360:672-674.
 26. Seubert, P., Oltersdorf, T., Lee, M.G., Barbour, T., Blomquist, C., Davis, D.L., Bryant, K., Fritz, L.C., Galasko, D., Thal L.J., Lieberburg, I., Schenk, D.B. (1993) Secretion of beta-amyloid precursor protein cleaved at the amino terminus of the beta-amyloid peptide. *Nature* 361:260-263.
 27. Johnson-Wood, K.L., Henriksson, T., Seubert, P., Oltersdorf, T., Lieberburg, I., Schenk, D.B. (1994) Identification of secreted beta-amyloid precursor binding sites on intact human fibroblasts. *Biochem. Biophys. Res. Comm.* 200:1685-1695.
 28. Seubert, P., Schenk, D. (1994) Recent advances in identifying markers of Alzheimer's disease. In: *Facts and Research in Gerontology, Volume 7.* (L.J. Fitten, ed.) Serdi, Paris, 31-37.
 29. Knops, J., Suomensaa, S., Lee, M., McConlogue, L., Seubert, P., Sinha, S. (1995) Cell-type and amyloid precursor protein-type specific inhibition of amyloid beta release by bafilomycin A1, a selective inhibitor of vacuolar ATPases. *J. Biol. Chem.* 270:2419-2422.

30. Vigo-Pelfrey, C., Seubert, P., Barbour, R., Blomquist, C., Lee, M., Lee, D., Coria, F., Chang, L., Miller, B., Lieberburg, I., Schenk, D., (1995) Elevation of microtubule-associated protein tau in the cerebrospinal fluid of patients with Alzheimer's disease. *Neurology* 45:788-793.
31. Seubert, P., Mawal-Dewan, M., Barbour, R., Jakes, R., Goedert, M., Johnson, G.V., Littersky, J.M., Schenk, D., Lieberburg, I., Trojanowski, J.Q., Lee, V.M.-Y. (1995) Detection of phosphorylated Ser262 in fetal tau, adult tau and paired helical filament tau. *J. Biol. Chem.* 270:18,917-18,922.
32. Motter, R., Vigo-Pelfrey, C., Kholodenko, D., Barbour, R., Johnson-Wood, K., Galasko, D., Chang, L., Miller, B., Clark, C., Green, R., Olson, D., Southwick, P., Wolfert, R., Munroe, B., Lieberburg, I., Seubert, P., Schenk, D. (1995) Reduction of beta-amyloid peptide42 in the cerebrospinal fluid of patients with Alzheimer's disease. *Ann. Neurol.* 38:643-648.
33. Games, D., Adams, D., Alessandrini, R., Barbour, R., Berthelette, P., Blackwell, D., Carr, T., Clemens, J., Donaldson, T., Gillespie, F., Guido, T., Hagopian, S., Johnson-Wood, K., Khan, K., Lee, M., Leibowitz, P., Lieberburg, I., Little, S., Masliah, E., McConlogue, L., Montoya-Zavala, M., Mucke, L., Paganini, L., Penniman, E., Power, M., Schenk, D., Seubert, P., Snyder, B., Soriano, F., Tan, H., Vitale, J., Wadsworth, S., Wolozin, B., Zhao, J. (1995) Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature* 373:523-527.
34. Haass, C., Lemere, C.A., Capell, A., Citron, M., Seubert, P., Schenk, D., Lannfelt, L., Selkoe, D.J. (1995) The Swedish mutation causes early-onset Alzheimer's disease by β -secretase cleavage within the secretory pathway. *Nature Med.* 1:1291-1296.
35. Seubert, P., Galasko, D., Boss, M.A. (1996) Use of CSF-based markers in the diagnosis of Alzheimer's disease. In: *Pharmacological Treatment of Alzheimer's Disease: Molecular and Neurobiological Foundations* (J.C. Brioni and M.W. Decker, eds.) John Wiley & Sons, Inc., New York, 345-366.
36. Littersky, J.M., Johnson, G.V.W., Jakes, R., Goedert, M., Lee, M., Seubert, P. (1996) Tau protein is phosphorylated by cAMP-dependent protein kinase and calcium/calmodulin protein kinase II within its microtubule-binding domains at Ser262 and Ser356. *Biochem. J.* 316:655-660.
37. Seubert, P. (1996) Commentary: Diagnosing Alzheimer's Disease: Tapping into new ideas. *Alzheimer's Disease Review* 1:84-86.

38. Citron, M., Diehl, T.S., Gordon, G., Biere, A.L., Seubert, P., Selkoe, D.J. (1996) Evidence that the 42- and 40-amino acid forms of amyloid β protein are generated from the β -amyloid precursor protein by different protease activities. *Proc. Natl. Acad. Sci. USA.* 93:13170-13175.
 39. Schenk, D., Lieberburg, I., Motter, R., Seubert, P., (1996) The effect of Apolipoprotein E genotype on biochemical markers of Alzheimer's disease. *Ann. New York Acad. Sci.* 802:92-100.
 40. Johnson, G.V.W., Seubert, P., Cox, T.M., Motter, R., Brown, J.P., Galasko, D. (1997) The tau protein in human cerebrospinal fluid in Alzheimer's disease consists of proteolytically derived fragments. *J. Neurochem.* 68:430-433.
 40. Citron, M., Westaway, D., Xia, W., Carlson, G., Diehl, T., Levesque, G., Johnson-Wood, K., Lee, M., Seubert, P., Davis, A., Kholodenko, D., Motter, R., Sherrington, R., Perry, B., Yao, H., Strome, R., Lieberburg, I., Rommens, J., Kim, S., Schenk, D., Fraser, P., St George Hyslop, P., Selkoe, D.J. (1997) Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid β -protein in both transfected cells and transgenic mice. *Nature Med.* 3:67-72.
 41. Galasko, D., Clark, C., Chang, L., Miller, B., Green, R.C., Motter, R., Seubert, P. (1997) Assessment of cerebrospinal fluid levels of tau protein in mildly demented patients with Alzheimer's disease. *Neurology* 48:632-635.
 42. Qiu, W.Q., Ye, Z., Kholodenko, D., Seubert, P., Selkoe, D.J. (1997) Degradation of amyloid beta-protein by a metalloprotease secreted by microglia and other neural and non-neural cells. *J. Biol. Chem.* 272:6641-6646.
 43. Johnson-Wood, K., Lee, M., Motter, R., Hu, K., Gordon, G., Barbour, R., Khan, K., Gordon, M., Tan, H., Games, D., Lieberburg, I., Schenk, D., Seubert, P., McConlogue, L. (1997) Amyloid precursor protein processing and A β 42 deposition in a transgenic mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA.* 94:1550-1555
 44. Xia, W., Zhang, J., Kholodenko, D., Citron, M., Podlisny, M.B., Teplow, D.B., Haass, C., Seubert, P., Koo, E.H., Selkoe, D.J. (1997) Enhanced production and oligomerization of the 42-residue amyloid β -protein by Chinese hamster ovary cells stably expressing mutant presenilins. *J. Biol. Chem.* 272:7977-7982.
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45. Podlisny, M., Citron, M., Amarante, P., Sherrington, R., Xia, W., Zhang, J., Diehl, T., Levesque, G., Fraser, P., Haass, C., Koo, E.H.M., Seubert, P., St George-Hyslop, P., Teplow, D.B., Selkoe, D.J. (1997) Presenilin proteins undergo heterogeneous endoproteolysis between thr291 and ala299 and occur as stable N- and C-terminal fragments in normal and Alzheimer brain tissue. *Neurobiol. Dis.* 3:325-337.
46. Gomez-Isla, T., Wasco, W., Pettingell, W.P., Gurubhagavatula, S., Schmidt, S.D., Jondro, P.D., McNamara, M., Rodes, L.A., DiBlasi, T., Growden, W.B., Seubert, P., Schenk, D., Growden, J.H., Hyman, B.T., Tanzi, R.E. (1997) A novel presenilin-1 mutation: increased beta-amyloid and neurofibrillary changes. *Ann Neurol.* 41:809-813.
47. Galasko, D., Chang, L., Motter, R., Clark, C.M., Kaye, J., Knopman, D., Thomas, R., Kholodenko, D., Schenk, D., Lieberburg, I., Miller, B., Green, R., Basherad, R., Kertiles, L., Boss, M.A., Seubert, P. (1998) High cerebrospinal fluid tau and low amyloid beta42 levels in the clinical diagnosis of Alzheimer disease and relation to apolipoprotein E genotype. *Arch Neurol.* 55:937-945.
48. Ellis, R.J., Seubert, P., Motter, R., Galasko, D., Deutsch, R., Heaton, R.K., Heyes, M.P., McCutchan, J.A., Atkinson, J.H., Grant, I. (1998) Cerebrospinal fluid tau protein is not elevated in HIV-associated neurologic disease in humans. *Neurosci Lett.* 254:1-4.
49. Xia, W., Zhang, J., Ostaszewski, B.L., Kimberly, W.T., Seubert, P., Koo, E.H., Shen, J., Selkoe, D.J. (1998) Presenilin 1 regulates the processing of beta-amyloid precursor protein C-terminal fragments and the generation of amyloid beta-protein in endoplasmic reticulum and golgi. *Biochemistry.* 37:16465-16471.
50. Lasser, R.A., Dukoff, R., Levy, J., Levin, R., Lehtimäki, T., Seubert, P., Sunderland, T. (1998) Apolipoprotein E epsilon 4 allele in association with global cognitive performance and CSF markers in Alzheimer's disease. *Int J Geriatr Psychiatry.* 13:767-774.
51. Schenk, D., Barbour, R., Dunn, W., Gordon, G., Grajeda, H., Guido, T., Hu, K., Huang, J., Johnson-Wood, K., Khan, K., Kholodenko, D., Lee, M., Liao, Z., Lieberburg, I., Motter, R., Mutter, L., Soriano, F., Shopp, G., Vasquez, N., Vandervert, C., Walker, S., Wogulis, M., Yednock, T., Games, D., Seubert, P. (1999) Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature.* 400:173-177.
52. Sunderland, T., Wolozin, B., Galasko, D., Levy, J., Dukoff, R., Bahro, M., Lasser, R., Motter, R., Lehtimäki, T., Seubert, P. (1999)

Longitudinal stability of CSF tau levels in Alzheimer patients. *Biol Psychiatry*. 46:750-755.

53. Larson, J., Lynch, G., Games, D., Seubert, P. (1999) Alterations in synaptic transmission and long-term potentiation in hippocampal slices from young and aged PDAPP mice. *Brain Res*. 840:23-35.
54. Sinha, S., Anderson, J.P., Barbour, R., Basi, G.S., Caccavello, R., Davis, D., Doan, M., Dovey, H.F., Frigon, N., Hong, J., Jacobson-Croak, K., Jewett, N., Keim, P., Knops, J., Lieberburg, I., Power, M., Tan, H., Tatsuno, G., Tung, J., Schenk, D., Seubert, P., Suomensaaari, S.M., Wang, S., Walker, D., Zhao, J., McConlogue, L., John, V. (1999) Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature*. 402:537-540.
55. Moriearty, P.L., Seubert, P., Galasko, D., Markwell, S., Unni, L., Vicari, S., Becker, R.E. (1999) Effects of time and cholinesterase inhibitor treatment on multiple cerebrospinal fluid parameters in Alzheimer's disease. *Methods Find Exp Clin Pharmacol*. 21:549-554.
56. Schenk, D.B., Seubert, P., Lieberburg, I., Wallace, J. (2000) Beta-peptide immunization: a possible new treatment for Alzheimer disease. *Arch Neurol*. 57:934-936.
57. Bard, F., Cannon, C., Barbour, R., Burke, R.L., Games, D., Grajeda, H., Guido, T., Hu, K., Huang, J., Johnson-Wood, K., Khan, K., Kholodenko, D., Lee, M., Lieberburg, I., Motter, R., Nguyen, M., Soriano, F., Vasquez, N., Weiss, K., Welch, B., Seubert, P., Schenk, D., Yednock, T. (2000) Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat Med*. 6:916-919.
58. Dovey, H.F., John, V., Anderson, J.P., Chen, L.Z., de Saint Andrieu, P., Fang, L.Y., Freedman, S.B., Folmer, B., Goldbach, E., Holtzman, E.J., Hu, K.L., Johnson-Wood, K.L., Kennedy, S.L., Kholodenko, D., Knops, J.E., Latimer, L.H., Lee, M., Liao, Z., Lieberburg, I.M., Motter, R.N., Mutter, L.C., Nietz, J., Quinn, K.P., Sacchi, K.L., Seubert, P.A., Shopp, G.M., Thorsett, E.D., Tung, J.S., Wu, J., Yang, S., Yin, C.T., Schenk, D.B., May, P.C., Alstiel, L.D., Bender, M.H., Boggs, L.N., Britton, T.C., Clemens J.C., Czilli, D.L., Dieckman-McGinty, D.K., Droste, J.J., Fuson, K.S., Gitter, B.D., Hyslop, P.A., Johnstone, E.M., Li, W.Y., Little, S.P., Mabry, T.E., Miller, F.D., Audia, J.E. (2001) Functional gamma-secretase inhibitors reduce beta-amyloid peptide levels in brain. *J Neurochem*. 76:173-181.
59. Games, D., Bard, F., Grajeda, H., Guido, T., Khan, K., Soriano, F., Vasquez, N., Wehner, N., Johnson-Wood, K., Yednock, T., Seubert, P.,

- Schenk, D. (2000) Prevention and reduction of AD-type pathology in PDAPP mice immunized with A β 1-42. *Ann N Y Acad Sci.* 920:274-284.
60. Bacskai, B.J., Kajdasz, S.T., Christie, R.H., Carter, C., Games, D., Seubert, P., Schenk, D., Hyman, B.T. (2001) Imaging of amyloid-beta deposits in brains of living mice permits direct observation of clearance of plaques with immunotherapy. *Nat Med.* 7:369-372.
61. Brayden, D.J., Templeton, L., McClean, S., Barbour, R., Huang, J., Nguyen, M., Ahern, D., Motter, R., Johnson-Wood, K., Vasquez, N., Schenk, D., Seubert, P. (2001) Encapsulation in biodegradable microparticles enhances serum antibody response to parenterally-delivered beta-amyloid in mice. *Vaccine.* 19:4185-4193.
62. Schenk, D., Seubert, P., Ciccarelli, R.B. (2001) Immunotherapy with beta-amyloid for Alzheimer's disease: a new frontier. *DNA Cell Biol.* 20:679-681.
63. Schenk, D., Games, D., Seubert, P. (2001) Potential treatment opportunities for Alzheimer's disease through inhibition of secretases and A β immunization. *J Mol Neurosci.* 22:259-267.
64. Bacskai, B.J., Kajdasz, S.T., McLellan, M.E., Games, D., Seubert, P., Schenk, D., Hyman, B.T. (2002) Non-Fc-mediated mechanisms are involved in clearance of amyloid-beta in vivo by immunotherapy. *J Neurosci.* 22:7873-7878.
65. Bard, F., Barbour, R., Cannon, C., Carretto, R., Fox, M., Games, D., Guido, T., Hoenow, K., Hu, K., Johnson-Wood, K., Khan, K., Kholodenko, D., Lee, C., Lee, M., Motter, R., Nguyen, M., Reed, A., Schenk, D., Tang, P., Vasquez, N., Seubert, P., Yednock, T. (2003) Epitope and isotype specificities of antibodies to beta-amyloid peptide for protection against Alzheimer's disease-like neuropathology. *Proc Natl Acad Sci USA.* 100:2023-2028.
66. Bussiere, T., Bard, F., Barbour, R., Grajeda, H., Guido, T., Khan, K., Schenk, D., Games, D., Seubert, P., Buttni, M. (2004) Morphological characterization of Thioflavin-S-positive amyloid plaques in transgenic Alzheimer mice and effect of passive A β immunotherapy on their clearance. *Am J Pathol.* 165:987-995.
67. Schenk, D., Hagen, M., Seubert, P. (2004) Current progress in beta-amyloid immunotherapy. *Curr Opin Immunol.* 16:599-606.
68. Masliah, E., Hansen, L., Adame, A., Crews, L., Bard, F., Lee, C., Seubert, P., Games, D., Kirby, L., Schenk, D. (2005) A β vaccination effects

on plaque pathology in the absence of encephalitis in Alzheimer disease. *Neurology*. 64:129-131.

69. Malinin, N.L., Wright, S., Seubert, P., Schenk, D., Griswold-Prenner, I. (2005) Amyloid-beta neurotoxicity is mediated by FISH adapter protein and ADAM12 metalloprotease activity. *Proc Natl Acad Sci USA*. 102:3058-3063.
 70. Masliah, E., Rockenstein, E., Adame, A., Alford, M., Crews, L., Hashimoto, M., Seubert, P., Lee, M., Goldstein, J., Chilcote, T., Games, D., Schenk, D. (2005) Effects of alpha-synuclein immunization in a mouse model of Parkinson's disease. *Neuron*. 46:857-868.
 71. Buttini, M., Masliah, E., Barbour, R., Grajeda, H., Motter, R., Johnson-Wood, K., Khan, K., Seubert, P., Freedman, S., Schenk, D., Games, D. (2005) Beta-amyloid immunotherapy prevents synaptic degeneration in a mouse model of Alzheimer's disease. *J Neurosci* 25:9096-9101.
 72. Lee, M., Bard, F., Johnson-Wood, K., Lee, C., Hu, K., Griffith S.G., Black, R.S., Schenk, D., Seubert, P. (2005) Abeta-42 immunization in Alzheimer's disease generates Abeta N-terminal antibodies. *Ann Neurol* 58:430-435.
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ABSTRACTS

1. Seubert, P.A., Hoang, L., Renosto, F., and Segal, I.H. (1983) ATP sulfurylase from *Penicillium chrysogenum*: the true specific activity and kinetic mechanism of the enzyme. Annual Meeting of the American Society of Biological Chemists, San Francisco.
2. Seubert, P.A., Baudry, M., Dudek, S., and Lynch, G. (1986) Calmodulin stimulates the degradation of brain spectrin by calpain. Annual Meeting of the Society for Neuroscience, Washington, D.C.
3. Seubert, P., Larson, J., Oliver, M., Jung, M.W., Baudry, M., and Lynch, G. (1988) Stimulation of NMDA receptors induces proteolysis of spectrin in hippocampus. Annual Meeting of the Society for Neuroscience, Toronto.
4. Seubert, P., Lee, K., and Lynch, G. (1989) Cerebral ischemia triggers NMDA receptor-linked cytoskeletal proteolysis of spectrin in hippocampus. Annual Meeting of the Society for Neuroscience, Phoenix.
5. Peterson, C., Vanderklisch, P., Seubert, P., Cotman, C., and Lynch, G. (1990) Increased spectrin breakdown in fibroblasts from aged and Alzheimer donors. Annual Meeting of the Society for Neuroscience, St. Louis.
6. Johnson-Wood, K.L., Henriksson, T., Seubert, P., Oltersdorf, T., Lieberburg, I., Schenk, D.B. (1990) Identification of specific binding sites for Iodine-125 secreted APP 751 on intact fibroblast cells. Second International Conference on Alzheimer's Disease and Related Disorders, Toronto.
7. Peterson, C., Vanderklisch, P., Seubert, P., Cotman, C., Lynch, G. (1990) Increased spectrin breakdown in fibroblasts from aged and Alzheimer donors. Annual Meeting of the Society for Neuroscience, St. Louis.
8. Sinha, S., Dovey, H.F., Seubert, P., Ward, P.J., Blacher, R.W., Blaber, M., Bradshaw, R.A., Arici, M., Mobley, W.C., Lieberburg, I. (1990) The Kunitz protease inhibitory domain of the beta amyloid precursor protein is sufficient for its protease inhibitory properties. Second International Conference on Alzheimer's disease and Related Disorders, Toronto.
9. Seubert, P., Oltersdorf, T., Lee, M.G., Barbour, R., Blomquist, C., Davis, D.L., Bryant, K., Galasko, D., Thal, L.V., Fritz, L., Lieberburg, I., Schenk, D. β -amyloid precursor protein is processed by an alternative potentially

amyloidogenic pathway. 1992 Annual Meeting of the Society for Neuroscience, Anaheim.

10. Seubert, P., Dovey, H.F., Blacher, R., Varghese, J., Colby, T., Lieberburg, I., Sinha, S. (1992) Identification of a major MET-ASP cleaving peptidase activity from human brain as metalloendopeptidase EC 3.4.24.15. Third International Conference on Alzheimer's Disease and Related Disorders, Abano Terme, Italy.
11. Seubert, P. (1993) Biomarkers of Alzheimer's disease: Beta-amyloid and tau. National Caregiving Foundation Symposium on "Progress in the Diagnosis of Alzheimer's disease," Rancho Mirage.
12. Vigo-Pelfrey, C., Seubert, P., Motter, R., Lieberburg, I., Butler, M., Lee, D., Barbour, R., Schenk, D. (1994) Detection of A β ₁₋₄₂ in CSF and various cell conditioned media. Annual Meeting of the Society for Neuroscience, Miami Beach.
13. Vigo-Pelfrey, C., Seubert, P., Lee, M., Lieberburg, I., Lee, D., Coria, F., Barbour, R., Schenk, D. (1994) Measurement of tau in human CSF and its possible utility in Alzheimer's disease diagnosis. Fourth International Conference on Alzheimer's Disease and Related Disorders, Minneapolis.
14. Younkin, S.G., Cai, X.-D., Suzuki, N., Cheung, T.T., Greenberg, B., Seubert, P., Lieberburg, I., Golde, T. (1994) Processing of normal and mutant amyloid β protein precursors. Fourth International Conference on Alzheimer's Disease and Related Disorders, Minneapolis.
15. Galasko, D., Hansen, L., Vigo-Pelfrey, C., Schenk, D., Seubert, P. (1995) Antemortem CSF tau is related to neuronal pathology at autopsy in Alzheimer's disease. Annual Meeting of the Society for Neuroscience, San Diego.
16. Haass, C., Lemere, C.A., Capell, A., Citron, M., Seubert, P., Schenk, D., Selkoe, D.J. (1995) β -secretase cleavage of β -amyloid precursor protein with the Swedish mutation occurs within the secretory pathway after the trans-golgi network. Annual Meeting of the Society for Neuroscience, San Diego.
17. McConlogue, L., Johnson-Wood, K., Tan, H., Gordon, M., Dovey, H., Lieberburg, I., Barbour, R., Games, D., Sinha, S., Schenk, D., Seubert, P., Samant, B. (1995) β APP processing and A β production in transgenic mouse brain. Annual Meeting of the Society for Neuroscience, San Diego.

18. Litersky, J.M., Seubert, P., Lee, M., Jakes, R., Goedert, M., Johnson, G.V.W. (1995) Tau is phosphorylated by cAMP-dependent protein kinase and calcium/calmodulin-dependent protein kinase II within the microtubule-binding domains at Ser262 and Ser356. Annual Meeting of the Society for Neuroscience, San Diego.
19. Vigo-Pelfrey, C., Motter, R., Kholodenko, D., Barbour, R., Johnson-Wood, K., Galasko, D., Chang, L., Miller, B., Clark, C., Green, R., Olson, D., Southwick, P., Wolfert, R., Munroe, B., Coria, F., Lieberburg, I., Seubert, P., Schenk, D. (1995) CSF levels of A β ₄₂ and tau are altered in Alzheimer's disease. Annual Meeting of the Society for Neuroscience, San Diego.
20. Sunderland, T., Peterson, R., Zubenko, G., Seubert, P. (1996) Biologic markers in Alzheimer's disease: Potential diagnostic and prognostic tests. Winter Conference on Brain Research, Snowmass.
21. Seubert, P., Motter, R., Schenk, D., Green, R., Chang, L., Miller, B., Clark, C., Galasko, D. (1996) Elevation of CSF tau in early stage Alzheimer's disease. Annual Meeting of the American Academy of Neurology, San Francisco.
22. Johnson-Wood, K., Lee, M., Motter, R., Hu, K., Gordon, G., Barbour, R., Gordon, M., Tan, H., Games, D., Lieberburg, I., Schenk, D., Seubert, P., McConlogue, L., (1996) The PDAPP mouse deposits β -amyloid₄₂ in a region-specific manner similar to Alzheimer's disease. Annual Meeting of the Society for Neuroscience, Washington D.C.
23. Dovey, H.F., Suomensaari, S., de St. Andrieu, P., Barbour, R., Sinha, S., Seubert, P., Schenk, D.B., Lieberburg, I., John, V. (1996) Alzheimer's amyloid precursor processing: Cathepsin D exhibits gamma-secretase activity. Annual Meeting of the Society for Neuroscience, Washington D.C.
24. Diehl, T.S., Citron, M., Gordon, G., Hartley, D., Seubert, P., Selkoe, D.J. (1996) Evidence that A β ₄₂ and A β ₄₀ are generated by different proteases. Annual Meeting of the Society for Neuroscience, Washington D.C.
25. Sunderland, T., Wolozin, B., Galasko, D., Levy, J., Motter, R., Dukoff, R., Bahro, M., Molchan, S., Rubinow, D., Lehtimäki, T., Seubert, P. (1996) Longitudinal stability of CSF tau levels in mid-stage Alzheimer patients. Annual Meeting of the Society for Neuroscience, Washington D.C.
26. Galasko, D.R., Brown, J., Clark, C., Chang, L., Miller, B., Green, R.C., Motter, R., Seubert, P. (1996) CSF tau is increased in very early stages of

Alzheimer's disease. Annual Meeting of the Society for Neuroscience, Washington D.C.

27. Seubert, P., Motter, R., Schenk, D., Lieberburg, I., Kholodenko, D., Galasko, D., Thomas, R., Chang, L., Miller, B., Clark, C., Knopman, D., Kaye, J., Green, R., Kertiles, L., Bashirzadeh, R., Boss, M. (1997) Apo E genotype influences the CSF level of A β 42 in Alzheimer's disease. Annual Meeting of the American Academy of Neurology, Boston, MA.
28. Galasko, D., Seubert, P., Motter, R., Schenk, D., Kholodenko, D., Lieberburg, I., Chang, L., Miller, B., Clark, C., Kaye, J., Camicioli, R., Knopman, D., Green, R., Thomas, R., Kertiles, L., Bashirzadeh, R., Boss, M.A. (1997) CSF levels of A β 42 and tau as aids to diagnosing Alzheimer's disease. Annual Meeting of the American Academy of Neurology, Boston, MA.
29. Seubert, P. (1997) CSF levels of tau and A β 42 as diagnostic markers of Alzheimer's disease. International Business Communications Sixth Annual Conference on Alzheimer's Disease, San Francisco, CA.
30. Xia, W., Zhang, J., Ostaszewski, B., Seubert, P., Koo, E., Selkoe, D. (1997) Mutant presenillins increase the generation of the 42-residue amyloid β peptide in the Golgi apparatus. Annual Meeting of the Society for Neuroscience, New Orleans, La.
31. Ellis, R., Seubert, P., Motter, R., Galasko, D., Heyes, M., Atkinson, J.H., Grant, I. (1997) Cerebrospinal fluid tau protein is not elevated in HIV neurologic disease. Annual Meeting of the Society for Neuroscience, New Orleans, La.
32. May, P.C., Boggs L.N., Calligaro, D., Seubert, P., Johnson-Wood, K., Chen, K., Games, D., Schenk, D., (1997) GFAP as a marker of plaque pathology in the PDAPP transgenic mouse. Annual Meeting of the Society for Neuroscience, New Orleans, La.